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# Bi-soft segment polyurethane membranes for Membrane Blood Oxygenators

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# Bi-soft segment polyurethane membranes for Membrane Blood Oxygenators*

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***Abstract*—Bi-soft segment polyurethane (PU) membranes containing poly(propylene oxide) as the first soft segment (SS) and different second SSs: poly(butadienediol) (PBDO), poly(dimethylsiloxane) (PDMS) and polycaprolactone (PCL) were prepared and characterized in terms of phase segregation, gas permeability and hemocompatibiliy with a view to their incorporation in membrane blood oxygenators (MBOs). Results show that a higher degree of mixing between microphases resulted in lower $CO_2$ permeabilities for the PU/PBDO and PU/PCL membranes. The PU/PDMS membranes present the highest permeability towards $O_2$ and $CO_2$ and phase segregation between the two SSs in not evident. The introduction of PCL as a second SS proved to be a major contribution to the enhancement of hemocompatibility. The versatility of the morphologies displayed by the addition of a second SS is an important asset in the design of novel and more efficient PU membranes for blood oxygenation. The feature of phase separation tailoring may bring insight into the transport mechanisms and the optimization of membrane performance both in terms of the permeation fluxes as well as hemocompatibility.**

## I. INTRODUCTION

Polyurethanes (PU) generally exhibit a two-phase structure in which hard segment (HS) enriched domains, composed mainly of the diisocyanate and/or the chain extenders, are dispersed in a matrix of soft segments (SS) composed of a sequence of polyol moieties. The hard-to-soft segment ratio can be changed by controlling the synthesis parameters in order to get tailored polyurethane membranes with improved bulk properties responsible for the final mass transfer properties [1] and surface properties that affect bio- and hemocompatibility [2] making them good candidates for extracorporeal blood circulation devices such as Membrane Blood Oxygenators (MBOs). The versatility of the structure design of polyurethane membranes can be further increased by the introduction of a second SS and was first reported by Zhao and de Pinho [3]. In these polymers the phase segregation that occurs between SS's and HS's may be accompanied by different extents of phase separation between the two SS's opening up new possibilities for tuning bulk and surface membrane properties [4]. With the aim of developing novel membranes for blood contacting devices, namely membrane blood oxygenators (MBOs), our group has focused on the synthesis of highly versatile bi-soft segment polyurethane PU membranes that ultimately fulfill a twofold goal: exhibit enhanced hemocompatibility and have suitable gas permeation rates. Achievements relative to these two points led to the synthesis of three groups of symmetric PU membranes containing poly(propylene oxide) (PPO) as the first SS and different second SS's: poly(butadienediol) (PBDO) defined as PU/PBDO membranes [5]–[7], poly(dimethylsiloxane) (PDMS) defined as PU/PDMS membranes [8] and polycaprolactone (PCL) defined as PU/PCL membranes [9]–[11]. The present work addresses the synthesis of three groups of bi-soft segment symmetric PU membranes: PU/PBDO, PU/PDMS and PU/PCL, each containing varying quantities of the second SS, with a view to their incorporation in future MBOs. The membranes were characterized in terms of phase segregation between HS's and SS's by Transmission Electron Microscopy (TEM) and Fourier transform infrared spectroscopy (ATR-FTIR) and the gas permeation properties towards oxygen ($O_2$) and carbon dioxide ($CO_2$) were measured by the constant pressure method. The blood compatibility properties in terms of hemolysis, thrombosis and platelet interaction of the membranes was evaluated by *in vitro* hemocompatibility assays using static rabbit blood incubation models.

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## II. EXERIMENTAL

### A. Materials

The PPO-based prepolymer (PUR, MW 3500) with three isocyanate terminal groups was supplied by Portuguese Hoechst, S.A. PBDO (MW 2800), PDMS (MW 5600), PCL diol (MW 530) and dibutyltin dilauryate (DBTDL) were supplied by Aldrich was used as catalysts. The pro-analysis solvent toluene supplied by Merck and other solvents: dimethyl formamide (DMF) and diethyl ether (DEE) were supplied by Aldrich.

### B. Synthesis of the symmetric PU/PBDO, PU/PDMS and PU/PCL membranes

The PU/PBDO membranes were prepared through the reaction of the isocyanate groups of PUR with the hydroxyl groups of PBDO, catalyzed by DBTDL at 70-80°C, under dry $N_2$ atmosphere [12]. The ratio of PUR and PBDO was varied to yield polymers with different compositions (100/0, 90/10, 75/25 e 40/60 wt%). The solutions were cast onto glass plates and heated at 70-80°C for 3.5 h. The films were then exposed to ambient air and curing by atmospheric moisture continued for at least 24 h. The PU/PDMS membranes were prepared through the same method by substituting PBDO for PDMS [8]. The ratio of PUR and PDMS was varied to yield polymers with different compositions (100/0, 90/10, 75/25 e 40/60 wt%) Finally, the PU/PCL membranes were prepared by the same method

using PCL-diol and PUR with the following PU to PCL diol wt % ratios: 100/0, 95/5, 90/10, and 75/25 [9].

### C. Phase-morphology characterization

The membranes prepared from casting solutions with different relative contents of the two SS's were analyzed with TEM for phase-morphology investigations. Osmium tetroxide ($OsO_4$) was used to selectively stain chemically unsaturated moieties and to provide contrast between the two soft segments. TEM images of the gold-coated PU/PBDO and PU/PDMS membranes were obtained by a Carl ZeissCEM902 TEM operated at an accelerating voltage of 80 kV. Before the imaging, the films were ultramicrotomed into 60-nm-thick sections and stained in $OsO_4$ vapor for 24 h [4], [8]. The molecular structure of the PU/PCL membranes was analyzed by ATR-FTIR spectroscopy (Perkin Elmer 1600 spectrometer). The ATR-FTIR measurements were made, at room temperature, on a KRS-5 crystal using a variable angle ATR unit (Groseby Specou Ltd.) at a nominal incident angle of 45º[11].

### D. Gas permeability

Gas permeation experiments were carried out for carbon dioxide and oxygen. The method used to measure the gas permeability was the constant pressure or variable volume method. For the measurements we built a system where a gas inlet and vent are connected to the bottom plate of the permeability cell while a flow meter is connected to the top plate. The feed pressure used ranged from 1 to 5 bar and the effective membrane surface area was 27.3 $cm^2$[12]. The gas permeability was determined by the following equation:

$$P = \frac{Vl}{(P_1 - P_2)At} \quad (1)$$

where $P$ is the gas permeability ($cm^3$(STP)$cmcm^{-2}s^{-1}cmHg^{-1}$), $V/t$ is the volumetric flow rate of the gas permeation, $l$ is the membrane thickness, $P_1$ And $P_2$ are the pressures and $A$ is the effective membrane area.

### D. Hemocompatibility

The hemocompatibility evaluation was carried out *in vitro* according to the ISO 10993-4:2002 standard[13]. All tests used pooled rabbit blood anticoagulated with ACD solution, at a blood/ACD ratio of 9:1.

Hemolysis was assessed following a method based on the ASTM F 756-00 [14]. Triplicate samples of each membrane were studied before and after extraction with phosphate-buffered saline (PBS) for 48 h at 37ºC, under static conditions. After 4 h of contact with static blood at 37ºC, the hemoglobin (Hb) released was quantified. From the Hb concentration released, the hemolysis index was calculated and expressed as a percentage in relation to the Hb concentration in the positive control (blood plus water), after subtracting a blank (blood plus PBS) from each Hb concentration. The membranes are classified according to the hemolysis index (HI) as non-hemolytic (HI 0–2%), slightly hemolytic (HI 2–5%), or hemolytic (HI >5%) [28].

Thrombosis was evaluated through a gravimetric assay

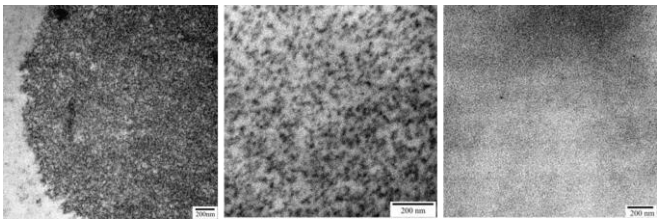

Figure 1. TEM micrographs of the PU/PBDO membranes containing (a) 10, (b) 25,and (c) 60wt % PBDO [4].

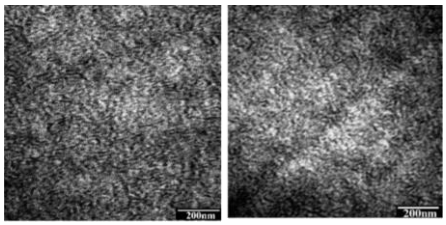

Figure 2. TEM micrographs of the PU/PDMS membranes containing (a) 25, (b) 75 wt % PDMS [8].

based on a version proposed by Imai and Nosé [15]. The thrombus mass formed on the top dense blood contacting surface of the PBS-extracted membranes was determined gravimetrically after different contact times, 15mins, 25mins, 35mins, 45mins and 55mins (one membrane sample, in triplicate, for each contact time) with recalcified, static blood. From the thrombus mass formed on each sample, a thrombosis degree was calculated as a percentage of the thrombus mass formed on the positive control (glass, evaluated in triplicate for each contact time) after subtracting the blank from each thrombus mass. A thrombosis degree of 100% was assigned to the positive control (glass).

Scanning Electron Microscopy (SEM) was used to visualize membrane surfaces of the PU/PCL membranes that had been in contact with platelet-rich plasma. PBS extracted were incubated for 30 min with 0.5 mL of PRP in 24-well polystyrene plates at 37ºC. Glass coverslips were used as a positive control. After incubation, the samples were rinsed three times with 1 mL portions of PBS, fixed with 1 mL of freshly prepared 1.5 vol % glutaraldehyde in PBS (30 min at room temperature) and, after further rinsing three times with 1 mL portions of PBS, postfixed with 1 mL of 0.05 vol % $OsO_4$ in PBS. The membrane surfaces were observed with a JEOL JSM T330 SEM (JEOL) at an accelerating voltage of 10 or 15 kV. Quantitative image analysis of representative micrographs was carried out using ImageJ. The indicators of platelet adhesion used were platelet deposition (PD; number of adhered platelets per 10,000 $\mu m^2$) and platelet coverage (PC; percentage of the sample area covered by platelets).

## III. Results and Discussion

### A. Phase-morphology characterization

Fig. 1 shows a series of TEM micrographs of the bulk morphology of the two-soft-segment urethane/urea polymers with different ratios of PU to PBDO. $OsO_4$ preferentially stained the unsaturated carbon linkages of the PBDO soft segments, and thus the dark regions in the TEM micrographs

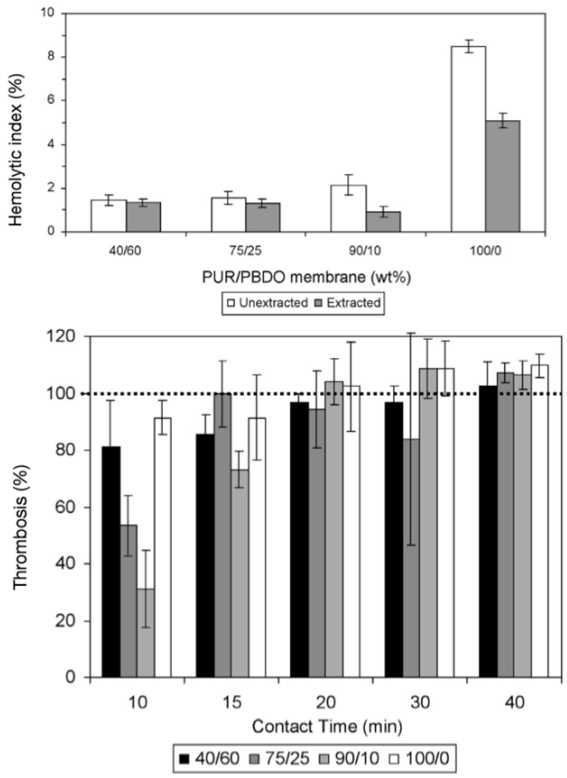


Figure 3. (a) Hemolytic index of the PU/PBDO membranes. (b) Percentage of thrombosis of the PU/PBDO membranes for different contact times with blood (glass: 100%, dotted line)[8].

correspond to PBDO domains. The membranes with PBDO concentrations lower than 60 wt % showed two-phase morphologies characteristic of phase separation. The micrographs of the membrane with 10 wt % PBDO (Fig. 1a) reveals microphase separation consisting of large domains richer in PBDO and scattered in a matrix richer in PPO. Fig. 1(b) shows the micrographs of the membrane with PBDO concentration of 25 wt % and a pronounced change in the morphology can be observed. The phase separation occurs now at the nanoscale, and the phase domains corresponding to this very fine morphology can be described as a disordered, wormlike domain structure. Fig. 1(c) presents a micrograph of a membrane with 60 wt % PBDO characterized by a single-phase morphology. An increase in the PBDO concentration leads to an increase in the mixing of the two soft segments, and this occurs through an evolution of the phase-separation morphology from a morphology composed of microscale domains to a morphology of nanoscale domains and finally to a single-phase morphology in which the two soft segments are molecularly mixed. Fig. 2 shows the micrographs of the bulk morphology of the membranes with 25 wt% (Fig. 2a) and 75 wt% (Fig. 2b) PDMS content. The siloxane regions, characterized by higher electron densities were more resistant to the transmittance of electrons and appear darker in the TEM images. The membranes show a fine co-continuous phase-separation structure without significant morphological dependence on PDMS content. ATR-FTIR analysis of the PU/PCL membranes for the membranes containing 0, 5 and 10wt% of PCL both urethane and urea groups are involved in hydrogen bonding while in in the membrane containing 15 wt.% PCL only the urethane groups seem to take part in hydrogen bonds. This suggests the presence of HS aggregates in all four membranes even though the total HS content decreases with the increase in PCL content. The aggregation of HSs is dependent on the ratio of the two SSs and increases with the increase of PCL content. It is concluded that the increase of PCL content reduces the freedom of the HS's allowing for less opportunities of mixing with the SS's [13].

### *B. Gas permeability*

Gas permeation measurements revealed that he $CO_2$ permeability of the PU/PBDO membranes was dependent on the ratio of PU to PBDO. Membranes containing 20 wt.% and 67 wt.% of PBDO had $CO_2$ permeability of 150 and 90 Barrer, respectively. In this case larger quantities of the second SS led to higher degrees of mixing between microphases which in turn resulted in lower $CO_2$ permeabilities[12]. With regard to the PU/PDMS membranes containing 25 wt.% to 75 wt.% of PDMS results showed evidence of phase separation between the two SS's. The structures analysis of the membranes containing lower amounts of PDMS showed that the HS's formed aggregates and that these decreased with the increase of PDMS content. Membranes containing 25 wt.% and 75 wt.% of PDMS had $CO_2$ permeability of 200 Barrer and 800 Barrer, respectively and $O_2$ permeability of 30 Barrer and 120 Barrer, respectively. The $CO_2$ and $O_2$ permeability of the membranes rose with the increase of PDMS. The high permeability of the 75 wt.% PDMS membrane was attributed to the higher content of siloxane groups, lower degree of cross-linking and lower aggregation of urethane/urea groups [8]. For the PU/PCL membranes, the $CO_2$ permeability coefficient varies with PCL content and was 188, 250, 337 and 113 Barrer for the membranes containing 0, 5, 10 and 15 wt.% PCL, respectively. For the membranes containing 0–10 wt.% of PCL it was found that the $CO_2$ permeability increases with the increase in PCL, with the decrease in HS% and increase in phase segregation. The $O_2$ permeabilities did not vary significantly with the PCL content and was approximately 10 Barrer for all of the PU/PCL membranes, 10–30 times lower than the $CO_2$ permeabilities.

### *C. Hemocompatibility*

Fig. 3a. shows that all PBS extracted and unextracted membranes with PBDO were non-hemolytic, while the membrane without PBDO was hemolytic. The differences between the membranes with PBDO were not statistically significant (ANOVA, P = 0.05). The difference between the unextracted and the PBS-extracted membranes decreased with an increase in PBDO content. Fig. 3b shows the percentage of thrombosis of the PUR/PBDO membranes for different contact times, taking thrombosis on glass as 100%. Except for the lowest blood contact time evaluated (10 min), the extent of clot formation on all membranes and on glass was similar. Thus, all membranes were thrombogenic for contact times above 10 min, with the exception of some membrane compositions for a contact time of 15 min. For a blood contact time of 10 min, the least thrombogenic membrane was that with 10% PBDO. This membrane and that with 25% PBDO were statistically less thrombogenic than the membrane without PBDO (ANOVA and Fisher's LSD test, P = 0.05), but the thrombogenicity of the 10%PBDO membrane was not significantly lower than that

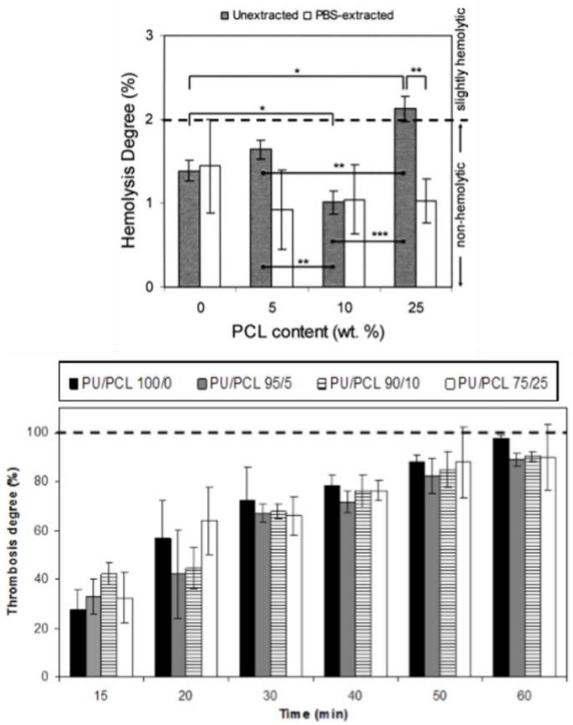


Figure 4. (a) Hemolytic index of the PU/PCL membranes. (b) Percentage of thrombosis of the PU/PCL membranes for different contact times with blood (glass: 100%, dotted line)[7].

of the 25% PBDO membrane, while both were significantly less thrombogenic than the 60% PBDO membrane. Although the presence of 10 and 25% of PBDO significantly decreased the thrombogenicity of this type of membrane, an increase in its content above these wt% seemed to increase their thrombogenicity. Fig. 4a shows that all PBS-extracted PU/PCL membranes were nonhemolytic for a contact time with blood of 3 h. Although statistically significant differences could be detected among some unextracted membranes, the significance disappeared after extraction with PBS. With the exception of the unextracted PU/PCL 75/ 25 membrane, which was slightly hemolytic (HI: 2.1%), the unextracted membranes were also nonhemolytic and did not vary regularly with PCL content.

In the only statistically significant difference between unextracted and PBS-extracted membranes (PU/PCL 75/ 25, a membrane with the lowest gel–sol fraction), extraction with PBS decreased considerably the HI. Fig. 4b shows that for short contact times with blood (15 min) the whole set of membranes showed thrombosis degrees of 27–42%. After a contact time of 60 min, the thrombosis degree of all the membranes reached that of the positive control. For contact times with blood between 15 and 60 min, the different membranes did not show significantly different thrombosis degrees (one-way ANOVA, $p > 0.05$). Fig. 5 shows SEM of the surfaces of PU/PCL membranes, after contact with PRP. Membranes without PCL showed high PD and PC, extensive platelet aggregation, and many platelets showed pseudopodia, indicating platelet activation. PU/PCL 90/10 showed few platelet aggregates and much lower PD and PC. In stark contrast, it was difficult to find any platelets adherent to PU/PCL 95/5 and to PU/PCL 75/25, and no activated platelets were found. This absence of platelet adhesion was

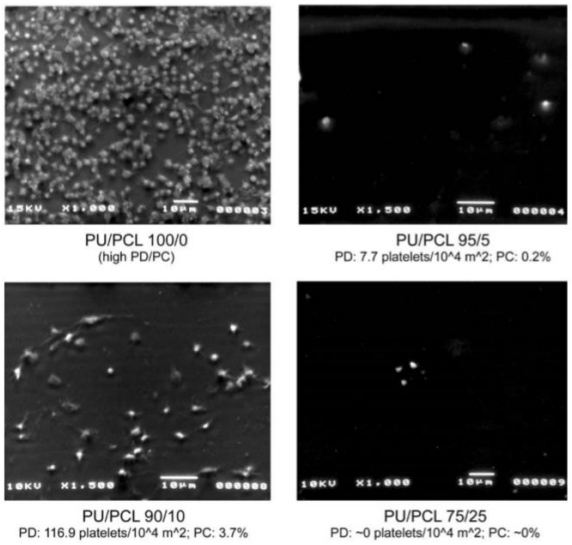


Figure 5. SEM microphotographs of the PU/PCL membranes after contact with platelets, and respective platelet deposition (PD) and platelet coverage (PC). In the PU/PCL 100/0 samples, quantitative image analysis could not be carried out due to the large degree of platelet aggregation[9].

coincident with a higher hard segment aggregation and with mixing between the two soft segments in the bulk. Platelet adhesion occurred for a membrane (PU/PCL 90/10) which had the lowest extent of hard segment aggregation, showed phase separation between the two soft segments in the bulk and had surface energy characteristics similar to those of PU/PCL 75/25, a membrane that, on the contrary, did not show platelet adhesion.

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